Antimicrobial Efficacy of Zinc Oxide Quantum Dots against *Listeria monocytogenes*, *Salmonella* Enteritidis, and *Escherichia coli* O157:H7

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ABSTRACT: Zinc oxide quantum dots (ZnO QDs) are nanoparticles of purified powdered ZnO. These were evaluated for antimicrobial activity against *Listeria monocytogenes*, *Salmonella* Enteritidis, and *Escherichia coli* O157:H7. The ZnO QDs were utilized as a powder, bound in a polystyrene film (ZnO-PS), or suspended in a polyvinylprolidone gel (ZnO-PVP). Bacteria cultures were inoculated into culture media or liquid egg white (LEW) and incubated at 22 °C. The inhibitory efficacies of ZnO QDs against 3 pathogens were concentration dependent and also related to type of application. The ZnO-PVP (3.2 mg ZnO/mL) treatment resulted in 5.3 log reduction of *L. monocytogenes* and 6.0 log reduction of *E. coli* O157:H7 in growth media after 48 h incubation, as compared to the controls. *Listeria* cells in the LEW control increased from 3.8 to 7.2 log CFU/mL during 8 d incubation, while the cells in the samples treated with 1.12 and 0.28 mg ZnO/mL were reduced to 1.4 and 3.0 log CFU/mL, respectively. After 8 d incubation, the cell populations of *Salmonella* in LEW in the presence of 1.12 and 0.28 mg ZnO/mL were reduced by 6.1 and 4.1 log CFU/mL over that of controls, respectively. ZnO powder and ZnO-PVP showed significant antimicrobial activities against all 3 pathogens in growth media and LEW. ZnO-PVP coating had less inhibitory effect than the direct addition of ZnO-PVP. No antimicrobial activities of ZnO-PS film were observed. This study suggested that the application of ZnO nanoparticles in food systems may be effective at inhibiting certain pathogens.

Keywords: liquid foods, nanoparticles, pathogens, zinc oxide

Introduction

Outbreaks of foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* continue to draw public attention to food safety. There is a need to develop new antimicrobials to ensure food safety and extend shelf life. The use of antimicrobial agents directly added to foods or through antimicrobial packaging is one effective approach.

In recent years, the use of inorganic antimicrobial agents in nonfood applications has attracted interest for the control of microbes (Okouchi and others 1995; Wilczynski 2000). Currently, most antibacterial inorganic materials are TiO_2 (Shirashi and others 1999; Huang and others 2000) and the ceramics immobilized antimicrobial metals, such as silver and copper (Kourai 1993; Wang and others 1995). Sawai and others (1995) have evaluated the antibacterial activity of 26 ceramic powders, and 10 were found to inhibit bacterial growth. Among these active powders, MgO, CaO, and ZnO exhibited strong antibacterial activity (Sawai and others 1995, 1998, 1999, 2000). It was found that the treatment with ZnO formulation caused a net reduction in bacterial cells of 78% and 62% in the case of treated cotton and cotton/polyester fabrics while the net reduction in fungi was calculated to be 80.7% and 32%, respectively (Zohdy and others 2003). Antibacterial activities of metal oxide (ZnO, MgO, and CaO) powders against *Staphylococcus aureus*, *E. coli*, or fungi were quantitatively evaluated in culture media (Sawai 2003; Sawai and Yoshikawa 2004).

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ZnO is 1 of 5 zinc compounds that are currently listed as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (21CFR182.8991). Zinc salt has been used for the treatment of zinc deficiency (Saldamli and others 1996; Lopes de Romana and others 2002). However, there are few data that demonstrate antimicrobial efficacy of ZnO in foods.

Nanotechnology has the potential to impact many aspects of food and agricultural systems. Food security, disease treatment delivery methods, new tools for molecular and cellular biology, new materials for pathogen detection, and protection of the environment are examples of the important links of nanotechnology to the science and engineering of agriculture and food systems (Weiss and others 2006). Nanoparticles have been reported for the application in nano sensor and nanotracer (Moraru and others 2003). Currently, there are very few reports related to the application of nanoparticles in food safety.

In the present study, ZnO QDs in the forms of powder, film (ZnO-PS), and gel (ZnO-PVP) were prepared and their antibacterial activities in culture media and liquid egg white were investigated. The objectives of this study were to evaluate different approaches, that is, direct/indirect additions of ZnO and potential applications of ZnO QDs at inhibiting 3 foodborne pathogens.

Materials and Methods

Preparation of ZnO QD samples

ZnO QD powder. Colloidal ZnO QDs were prepared and purified according to our previous report (Sun and others 2007). Briefly, 200 mL of methanol containing 0.08 M of KOH (99.99%, Aldrich, Milwaukee, Wis., U.S.A.) were heated at 60 °C for 30 min to obtain a homogeneous solution. Powder containing 0.008 mol $Zn(AC)_2 \cdot H_2O$

doi: 10.1111/j.1750-3841.2008.01013.x

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Journal of Food Science
A Publication of the Institute of Food Technologists

(99%, Fluka, Castle Hill, Sweden) was then directly added into a basic methanol solution to make the final [Zn^{2+}] of 0.04 M. This starting solution was allowed to react at 60 °C for 2 h with stirring and refluxing. After reaction, the ZnO solution was concentrated 10 times via rotary evaporation under vacuum at 40 °C. Subsequently, hexane and isopropanol were added into the concentrated ZnO solution with a volume ratio hexane:isopropanol:methanol of 5:1:1. The ZnO QDs precipitated immediately after adding hexane and isopropanol. This mixture was kept at 0 °C overnight until the ZnO QDs were fully precipitated and settled to the bottom of the container. After the removal of the supernatant, ZnO QDs were redispersed in methanol. The above-mentioned operations were repeated at least twice to purify the ZnO nanoparticles. The purified ZnO QDs were then baked in an oven at 110 °C for 2 h and white ZnO QD powder was obtained.

Figure 1 shows the high-resolution transmission electron microscopy (HR-TEM) image of prepared ZnO QDs. The spherical particles were highly crystalline (insert of the TEM image) and had an average uniform size distribution of 5 nm. After concentrating and washing, zinc by-product (zinc layered double hydroxide) and unwanted ions (K^+ and AC^-) were effectively removed (>99.8% of K^+ can be removed in 3 washes) (Sun and others 2007). The prepared ZnO QDs were directly used to study the antimicrobial activity of ZnO QD powder after drying.

Polystyrene/ZnO (PS/ZnO) nanocomposite films. Polystyrene was first dissolved in toluene. The purified ZnO nanoparticles were redispersed into methanol and then directly added into the PS toluene solution. Two concentrations of 0.01 and 1.0 wt% of ZnO QDs in PS were prepared. The solution mixtures were then cast into glass Petri dishes and the solvents were evaporated in a vacuum oven at room temperature. The PS/ZnO nanocomposite films were obtained after evaporation and then heated at 80 °C to remove any solvents trapped in the polymer. For comparison purposes, a neat PS film without ZnO QDs was also prepared according to the above-mentioned method.

PVP-capped ZnO (ZnO-PVP) QDs. PVP-capped ZnO QDs were prepared by hydrolyzing $Zn(AC)_2{\cdot}H_2O$ in a basic methanol solution with the presence of PVP. The synthesis and purification were the same as the colloidal procedure described previously, except that 1.332 g of PVP (MW 8000, Sigma-Aldrich, St. Louis, Mo., U.S.A.) were also dissolved in methanol containing KOH before the reaction. After purification, the ZnO-PVP QDs were redispersed in methanol again and then the methanol and remaining solvents were fully removed via rotary evaporation under vacuum to avoid any contamination. Five milliliters of deionized and distilled water were added into the dried PVP-ZnO QDs. After ultrasonication, a gel-like sample containing ZnO-PVP QDs was obtained.

The HR-TEM image shows that the ZnO-PVP QDs were highly crystalline and had a uniform size distribution (Figure 2). The particle size was estimated to be around 4 nm, which was slightly smaller than the ZnO QDs prepared without PVP.

Bacterial inhibition tests

Cultures. *Listeria monocytogenes* Scott A 724, *Escherichia coli* O157:H7 Oklahoma, and *Salmonella enterica serovar* Enteritidis ATCC 13076 were obtained from the culture collection of the U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Research Center. Stock cultures were maintained at −80 °C. The stains were propagated on Tryptic Soy Agar (TBA: Difco Laboratories, Detroit, Mich., U.S.A.) at 37 °C and maintained at 0 to 2 °C until use. Prior to the inoculum preparation, *E. coli* O157:H7 and *S.* Enteritidis cells were grown in tryptic soy broth (TSB: Remel Inc., Lenexa, Kans., U.S.A.); while *L. monocytogenes* cells were grown in brain heart infusion broth (BHIB: Difco Laboratory). Cultures were incubated aerobically at 37 °C for 16 to 18 h.

Liquid culture test. Commercial pasteurized liquid egg white was purchased from a local grocery store. The pH of liquid egg white was 8.0 to 8.2. ZnO powder, ZnO film, or ZnO-PVP was placed in a 15-mL glass tube (culture medium test) or a 50-mL glass bottle (liquid food test) with a medium (BHIB, TSB, or liquid egg white) and inoculated with overnight cultures of *L. monocytogenes*, *E. coli* O157:H7, or *S.* Enteritidis. The glass tubes and the glass bottles contained 10 and 25 mL of liquid, respectively. The final cell density in a medium was approximately 1×10^4 cells per milliliter in each bacterial inhibition test. The tubes or bottles were shaken at 50 rpm at 22 ± 1 °C. The inoculated media were sampled (1.0 mL) at certain time intervals. Specimens were serially diluted by sterile Butterfield's phosphate buffer (pH 7.2, Hardy Diagnostics, Santa Maria, Calif., U.S.A.), then pour plated onto BHI agar or tryptic soy agar (TSA: Difco). Plates were incubated at 37 °C for 24 h. Zinc oxide-free inoculated medium served as a control. Room temperature (22 °C) was selected for the incubation tests after considering the worst case scenario in which food was left at room temperature, at which the pathogens grow much faster, rather than refrigerated.

Agar diffusion test. The agar diffusion test was especially used for film samples. The inoculum of *L. monocytogenes*, *E. coli*

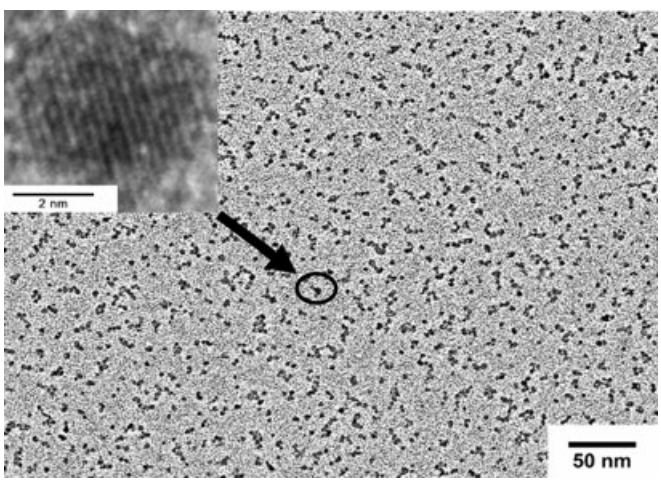

Figure 1—HR-TEM image of ZnO QD powder.

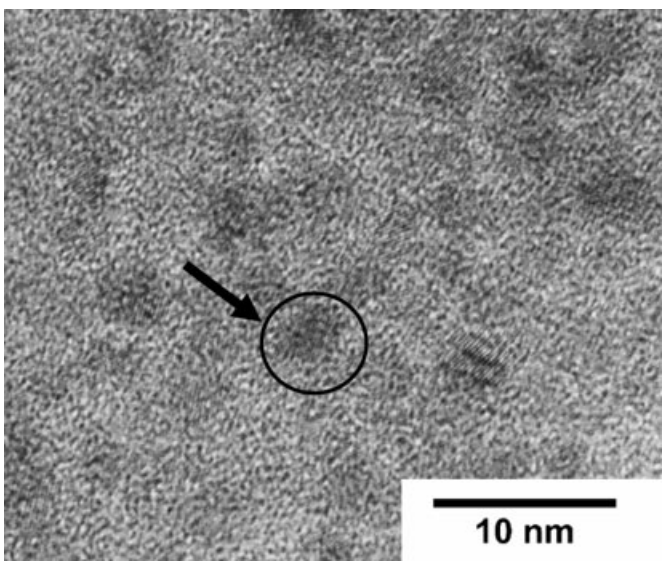

Figure 2—HR-TEM image of PVP-ZnO QD film.

O157:H7, or *S.* Enteritidis was spread on the surface of BHIA (*L. monocytogenes*) or TSA (*E. coli* O157:H7 and *S.* Enteritidis) plates, to produce a lawn containing approximately 10^5 CFU/cm^2. Each film sample was placed on surface-inoculated BHI agars and incubated at 37 °C for 24 h. Inhibition zone around film specimens was used to indicate antibacterial activity of each film sample.

Coating/release test. ZnO-PVP was coated around the inside surface of a glass tube (10 mm in diameter) about 1-cm-wide ring below the top level of medium, as illustrated in Figure 3. After each sampling (1 mL), the tube was refilled with an equal amount of fresh sterile medium to maintain constant volume/level of the medium during the testing period. The weights of tubes with ZnO coating were recorded before and after incubation so that the weight loss of ZnO coating into a medium could be calculated. Dilution and plating were the same as in the liquid culture test.

Scanned electron microscopy (SEM)

ZnO-PS films were cut with surgical scissors into 3 × 5 mm pieces and mounted directly on specimen stubs with 2-sided adhesive tabs of Carbon (Electron Microscopy Sciences, Hatfield, Pa., U.S.A.). Mounted film strips were sputter coated with a thin layer of gold using a Scancoat Six Sputter Coater (BOC Edwards, Wilmington, Mass., U.S.A.). Digital images of topographical features of the film strips were collected using a Quanta 200 FEG environmental scanning electron microscope (FEI Co., Inc., Hillsboro, Oreg., U.S.A.) operated in the high vacuum/secondary electron imaging mode at an accelerating voltage of 10 kV.

High-resolution transmission electron microscopy (HR-TEM)

The purified ZnO QDs and ZnO-PVP QDs were redispersed in methanol, diluted and droplets of each solution were placed onto a 400-mesh carbon coated copper grid. The grids were then dried in a desiccator for 1 d before imaging. HR-TEM of the above-mentioned samples was carried out using a JEOL 2010 high-resolution transmission electron microscope operated at 200 kV.

Statistical analysis

Antimicrobial experiments were conducted in triplicate. Data points were expressed as the mean ± SD. Data were analyzed using analysis of variance (ANOVA) from SAS version 9.1 software (SAS Inst., Inc., Cary, N.C., U.S.A.). Duncan's multiple range tests were used to determine the significant difference of mean values. Unless stated otherwise, significance was expressed at 5% level.

Results and Discussion

Antibacterial activity in microbial culture media

Figure 4 shows the effect of ZnO treatment on the growth of *L. monocytogenes* in BHI broth at 22 °C. Both treatments of ZnO powder and ZnO-PVP exhibited significant inhibitory effect on the growth of *L. monocytogenes* during 48 h incubation, as compared to the control. Among the two, the treatment of ZnO-PVP was the most effective in bacterial inhibition. The cell populations of *L. monocytogenes* in BHI broth with ZnO-PVP were 2 log CFU/mL at 8 h, 3 log CFU/mL at 24 h, and 3.7 log CFU/mL at 48 h, while the control values were 5.5, 8.6, and 9.0 log CFU/mL, respectively.

From the coating test, as shown in Figure 4, the treatment of ZnO-PVP coating had significantly less *L. monocytogenes* cells than the control during incubation at 22 °C. However, the ZnO-PVP coating had less inhibitory effect than the direct addition of ZnO-PVP. It could be explained that in ZnO-PVP coating, fewer ZnO nanoparticles were available in the medium. Approximately 15% of the ZnO-PVP coating was lost into the liquid medium during 48 h incubation. In other words, only one-fifteenth of the ZnO was available in the medium to play a role of inhibition as compared to the direct addition of ZnO-PVP. These data suggested that the antibacterial activity of ZnO was concentration dependent.

The treatment with ZnO-PS film had no effect on suppressing the growth of *L. monocytogenes* in the liquid culture test (Figure 4), which was further confirmed by the agar diffusion test. Figure 5 indicates the absence of zones of inhibition around the films containing 0.01% or 1% ZnO QDs. Similar results were also observed for *E. coli* 0157:H7 and *S.* Enteritidis (data not shown). These data

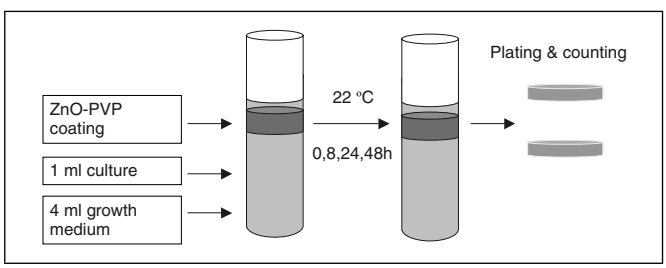

Figure 3—Diagram of ZnO coating procedures.

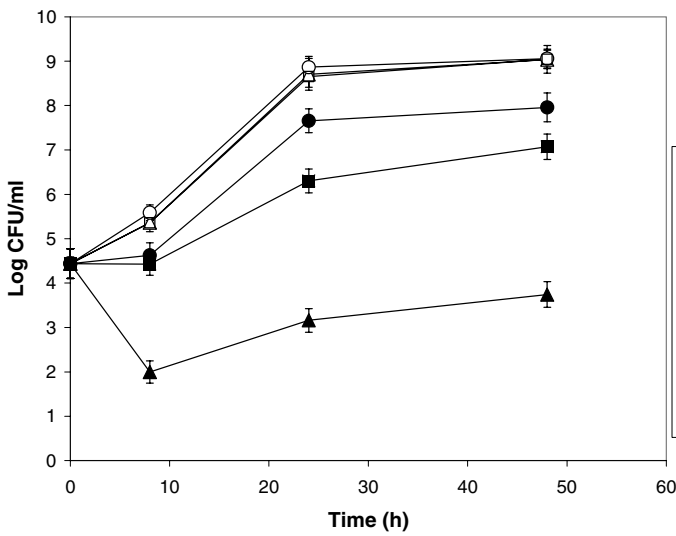

Figure 4—Effect of ZnO nanoparticles and ZnO-PVP coating on growth of *L. monocytogenes* in BHIB at 22 °C. ZnO-PS film: 3 mg/mL with 1% ZnO; ZnO-PVP: 40 mg ZnO-PVP/mL with equivalent to 3.2 mg ZnO/mL; ZnO powder: 10 mg/mL; PVP powder: 37 mg/mL. 6 mg ZnO-PVP/mL coating released to BHIB with equivalent to 0.48 mg ZnO/mL. Error bars represent the standard deviation of the mean.

implied that ZnO was not released from the film into the growth broth or agar. The SEM image (Figure 6) reflected the smooth surface of the ZnO-PS film. It suggested that the ZnO molecules were tightly bound within the film, which prevented ZnO release and expression of the antimicrobial action.

To further investigate the effect of ZnO concentration on antibacterial activity, 3 ZnO-PVP concentrations were tested. Figure 7 shows the effect of ZnO-PVP concentration on the growth of *L. monocytogenes* in BHI broth. Each test tube contained ZnO nanoparticles equivalent to 0, 0.1, 0.3, and 0.5 mg ZnO per mL of medium, respectively. The treatment with 0.1 mg ZnO per mL was not statistically different from that of the control. Increasing the ZnO concentration resulted in more bacterial inhibition. The differences of cell counts between the control and 0.5 mg/mL treatment were 1.8, 5.2, and 3.5 log CFU/mL at 8, 24, and 48 h, respectively. The treatment with 0.3 mg/mL showed significant effects at 8 and 24 h, but not at 48 h. The PVP treatment (0 mg ZnO/mL) had no inhibitory effect.

Figure 8 illustrates the effect of ZnO on the growth of *E. coli* O157:H7 in TSB culture medium at 22 °C. Similar to *L. monocytogenes*, ZnO-PVP showed strongest antibacterial activity against *E. coli* O157:H7. The cell populations treated by ZnO-PVP were maintained at a level of 3 log CFU/mL during 48 h incubation whereas the control and PVP alone increased to 9 log CFU/mL.

The ZnO powder was less effective against *L. monocytogenes* (Figure 4) or *E. coli* O157:H7 (Figure 8) as compared with ZnO-PVP, even though the treatment of ZnO powder had higher ZnO concentration (10 mg ZnO/mL) than that of ZnO-PVP treatment (3.2 mg ZnO/mL). When ZnO powders were used, ZnO particles were visually observed on the bottom of tubes during incubation because of ZnO sedimentation, which probably is the reason for their lower antibacterial activity. The above-mentioned results suggested that it was essential for ZnO molecules to contact or penetrate into microbial cells to express their antibacterial activities. These data might also be interpreted as a requirement for interaction of ZnO with the bacterial cell wall or membrane for expression of antibacterial activity. Further study is needed to confirm the above conjecture.

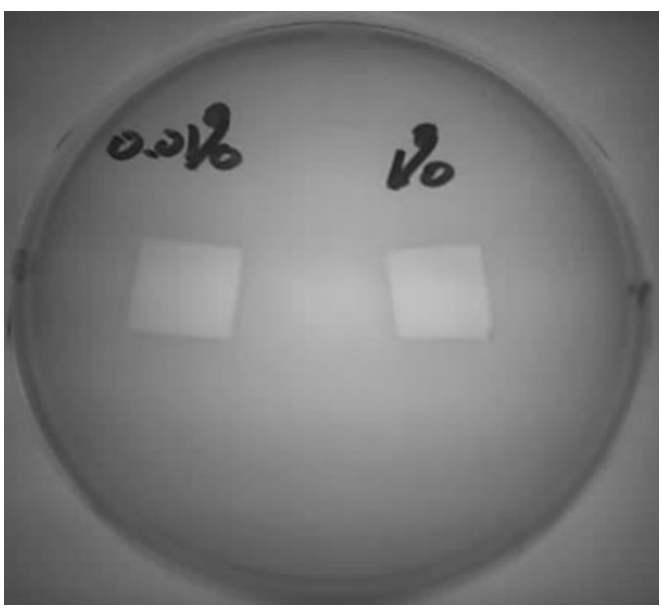

Figure 5—Agar diffusion test of ZnO-PS films showing no inhibition zone around the films. Left: PS film with 0.01% ZnO; Right: PS film with 1.0% ZnO.

PVP is a food additive listed in 21CFR 173.55. In our study, PVP alone showed no antibacterial activities against *L. monocytogenes* (Figure 4 and 7) or *E. coli* O157:H7 (Figure 8). These data suggest that the antibacterial activity of ZnO-PVP was contributed solely from ZnO nanoparticles, not from PVP. Therefore, treatment with PVP alone was excluded from further experiments.

The inhibition action of ZnO in microbial culture media may come from 2 parts: killing and growth suppression. At higher ZnO concentration (ZnO-PVP in Figure 4 and 8), ZnO treatments initially kill a sensitive subpopulation of cells and reduce the total microbial population and then retard the growth by a resistant surviving subpopulation. At lower ZnO concentration such as ZnO powder and ZnO coating in Figure 4 and ZnO-PVP in Figure 7, ZnO treatments only suppress the growth of bacteria and result in an 8 or 24 h lag time before a slow growth.

Application of ZnO nanoparticles in food safety

Liquid egg white, *S.* Enteritidis, and *L. monocytogenes* were selected for this study because both the physical and functional properties of liquid egg products including liquid egg white are sensitive to thermal treatments. The high thermal sensitivity of egg components prevents the application of more intense heat treatments. Therefore, it would be desirable to use new antimicrobial agents or other nonthermal processing techniques to ensure the safety of liquid egg products while their desired qualities are not altered. Furthermore, occurrences of egg-related outbreaks of *Salmonellosis* (CDC 2001) and potential outbreaks from egg contamination of *L. monocytogenes* (Leasor and Foegeding 1989; Moore and Madden 1993) have heightened the concern for the safety of egg-related products.

Two concentrations of ZnO QDs were used as the antimicrobial treatments in liquid egg white samples: 0.28 and 1.12 mg/mL. Figure 9 illustrates the effect of ZnO against growth of *L. monocytogenes* in liquid egg white during 8 d storage at 22 °C. After an initial drop from 3.9 to 3 log CFU/mL at 8 h, *L. monocytogenes* cells in the control increased to 7.2 log CFU/mL, while the cells in the liquid egg white sample treated with 0.28 mg ZnO/mL remained at 3 log CFU/mL. The *L. monocytogenes* cells in the samples treated with 1.12 mg ZnO/mL were reduced to 1.4 log CFU/mL after 8 d. Depending on the concentration, ZnO QDs could significantly inhibit

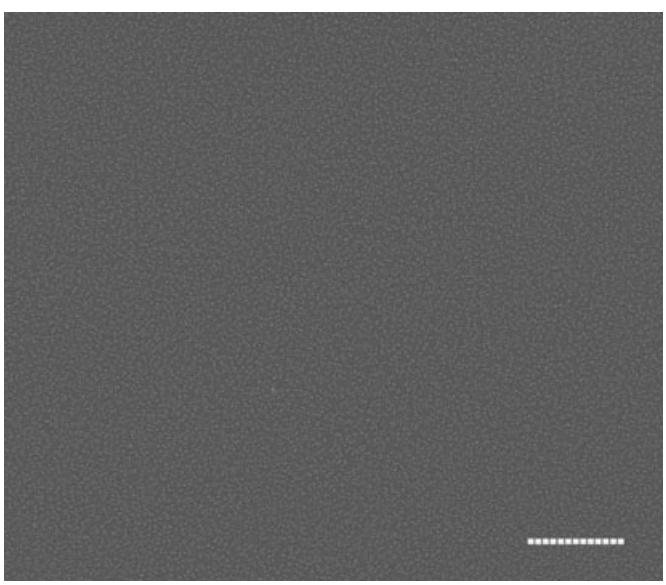

Figure 6—SEM microphotograph of ZnO-PS film. The bar represents 2.0 μm.

or reduce *L. monocytogenes* in liquid egg white. These findings imply that the egg protein assisted in dispersal of ZnO nanoparticles so that the treatment efficacy was more like that obtained with ZnO-PVP in the 1st set of experiments (Figure 4).

Figure 10 shows the growth of *S.* Enteritidis in liquid egg white with or without the presence of ZnO QDs at 22 °C. The numbers of *Salmonella* cells in liquid egg white samples were significantly reduced in the presence of ZnO. Similar to the case

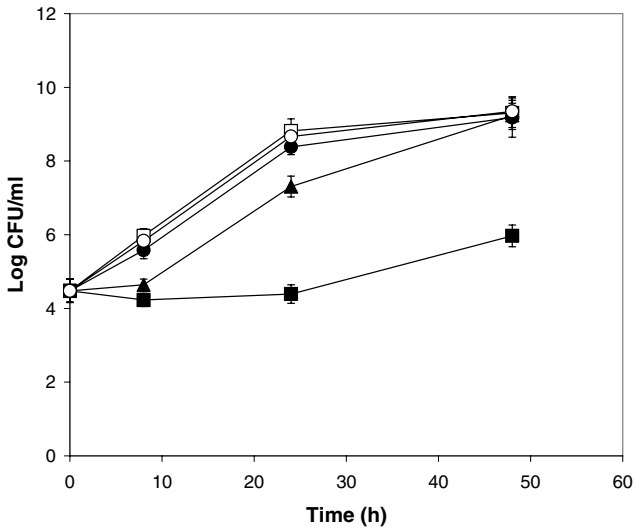

Figure 7—Effect of ZnO concentration on growth of *L. monocytogenes* in BHIB at 22 °C. Error bars represent the standard deviation of the mean.

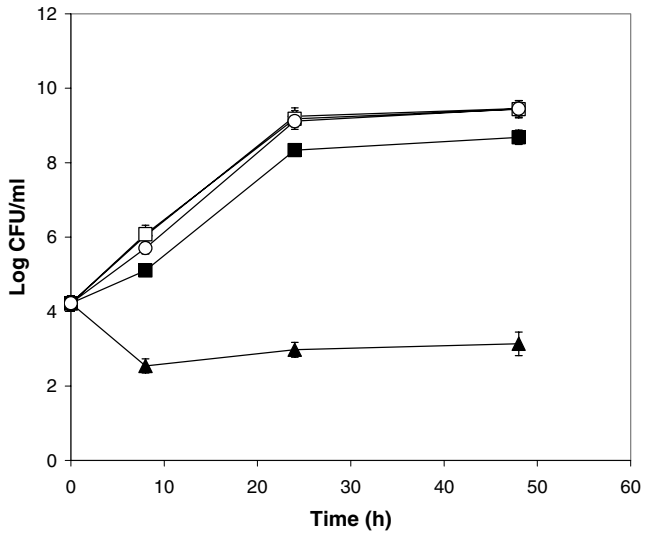

Figure 8—Effect of ZnO nanoparticles on growth of *E. coli* O157:H7 in TSB at 22 °C. ZnO-PS film: 3 mg/mL with 1% ZnO; ZnO-PVP: 40 mg ZnO-PVP/mL with equivalent to 3.2 mg ZnO/mL; ZnO powder: 10 mg/mL; PVP powder: 37 mg/mL. Error bars represent the standard deviation of the mean.

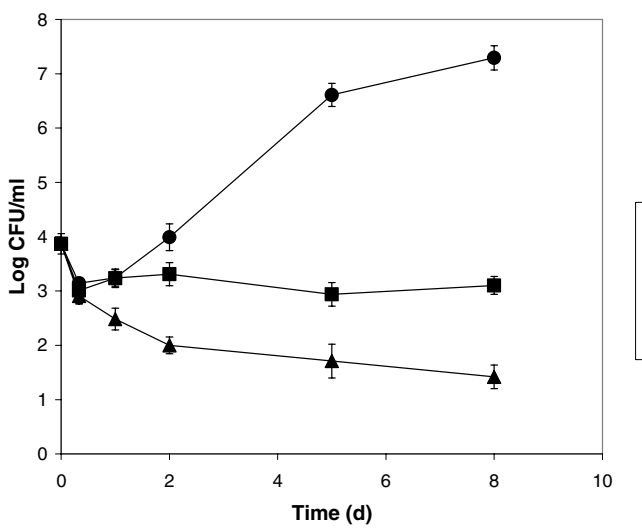

Figure 9—Effect of ZnO nanoparticles on growth of *L. monocytogenes* in liquid egg white at 22 °C. Error bars represent the standard deviation of the mean.

of *L. monocytogenes*, the inhibitory effect of ZnO nanoparticles against *Salmonella* was dependent on the concentration of ZnO. As shown in Figure 10, after initial killing of cells by ZnO treatment, the cell populations of *Salmonella* remained constant during the 8 d incubation with the numbers of cells after 8 d at 5.5 log CFU/mL for 0.28 mg/mL. In the 1.12 mg/mL solution, *Salmonella* cells decreased to 3.5 log CFU/mL, whereas the control grew to 9.7 log CFU/mL.

It was noticed that both *Listeria* and *Salmonella* grew in the control samples that contain native lysozyme, a natural antimicrobial (Figure 9 and 10). Gram-negative bacteria are less susceptible to the action of lysozyme than Gram-positive bacteria. For *Listeria*, this could be explained that small amount of lysozyme natively found in egg white may not be enough to prevent growth of highly inoculated *Listeria* cells. Branen and Davidson (2004) discovered that lysozyme alone inhibited growth of *L. monocytogenes* Scott A, but did not inhibit growth of other *L. monocytogenes* strains or any of the tested Gram-negative strains. Hughey and Johnson (1987) reported that four strains of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella typhimurium* were not inhibited by lysozyme (200 μg/mL) in BHI broth. Payne and others (1994) also found that lysozyme (100 to 200 μg/mL) had no effect on the growth of *L. monocytogenes*, *E. coli* O157:H7, *S. typhimurium*, or *P. fluorescens*.

Similar to ZnO in microbial culture media, increasing ZnO concentration in a food system (liquid egg white) resulted in more killing of a sensitive subpopulation of bacterial cells, longer lag time, and less growth of a resistant surviving subpopulation. The mechanism for killing and growth suppression induced by ZnO nanoparticles has not been reported yet and needs further investigation. Several studies show that zinc homeostasis is regulated through a number of specific and nonspecific membrane-bound uptake and efflux pumps (Blencowe and Morby 2003). Additionally, zinc is capable of inhibiting nitric oxide formation (Abou-Mohamed and others 1998) and prevents sulfhydryl groups from oxidation (Lim and others 2004).

Our results revealed that the uniform distribution or dispersion of ZnO in a liquid medium (culture medium or liquid egg white) was critical to increase the efficiency of microbial inhibition. Availability of ZnO in a medium would be more important than its total concentration used, as in the case of ZnO powder compared with ZnO-PVP. ZnO sedimentation in a liquid system should be reduced or avoided. Nanotechnology provides an opportunity to solve this problem. Because of their small size and large surface area, the nanoparticles possess (1) ability to penetrate through cell membranes and ensure rapid antibacterial activities, (2) uniform distributed within a medium with low sedimentation, and (3) minimal quantity required to be effective per volume of food.

The recommended maximum dietary allowance for zinc was 40 mg per day for adult (NIH 2007), which is equivalent to 143 mL of liquid egg white daily intake if 0.28 mg of ZnO per mL of liquid egg white is used. Therefore, further research is necessary to investigate the efficacy of ZnO at a lower concentration level to inactivate pathogens in foods. The combined usage of ZnO with another antimicrobial agent, such as nisin, may be an effective approach to reduce the amount of ZnO used per volume of food while maintaining or increasing its effectiveness in microbial inactivation.

Conclusions

ZnO nanoparticles possess antimicrobial activities against *L. monocytogenes* and *S.* Enteritidis in liquid egg white and culture media, and *E. coli* O157:H7 in culture media. The inhibitory effect was concentration dependent. The higher the concentration of ZnO used, the higher the antibacterial effect was achieved. This study compared the effectiveness of ZnO in powder, PVP-capped, film, and coating forms. The availability of ZnO QDs in media was important for antibacterial efficiency. The results of this study demonstrated several approaches (powder, PVP capped, film, and coating) for incorporation of ZnO into food system. This is a preliminary study that provides a starting point to determine whether the use of ZnO nanoparticles has a potential in food safety. Further research will be required to establish the parameters for optimal antimicrobial effect. Parameters such as concentrations, times, temperatures, and combination with other bacteriocins (synergistic effects) will be the focus of further study.

Acknowledgments

The authors wish to thank Dr. Gerald Sapers and Dr. Joshua Gurlter for their thoughtful reviews of this article, Dr. William Dantzer for valuable discussion, and Anita Parameswaran, Peter Cooke, and Guoping Bao for their technical assistance.

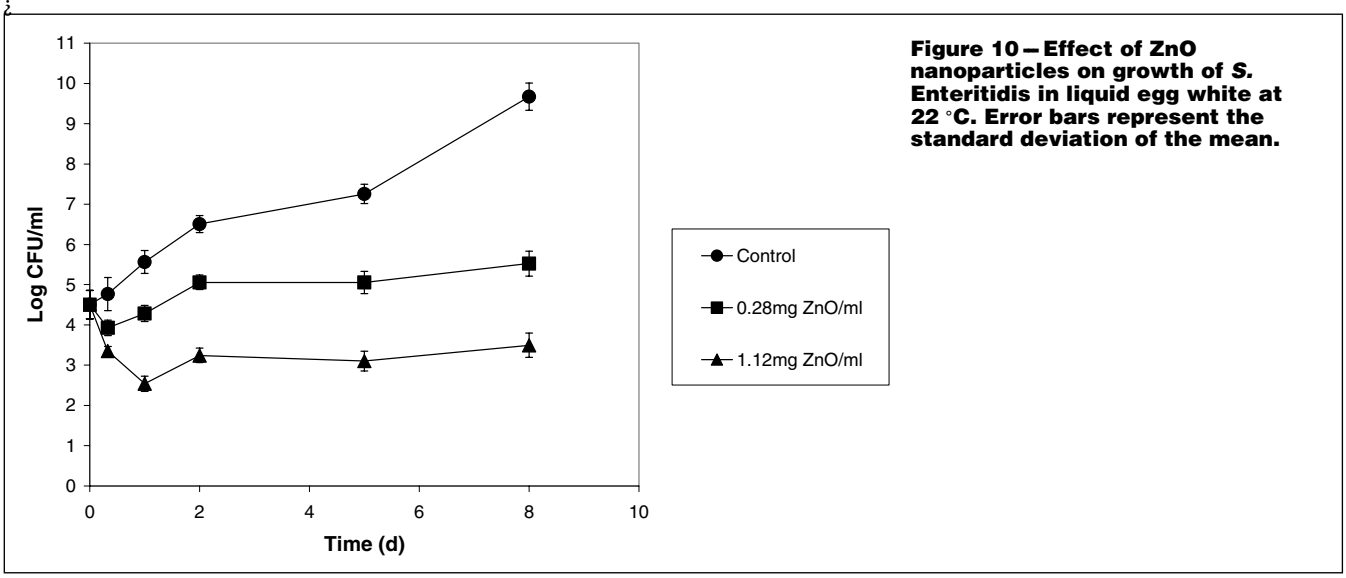

Figure 10—Effect of ZnO nanoparticles on growth of *S.* Enteritidis in liquid egg white at 22 °C. Error bars represent the standard deviation of the mean.

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